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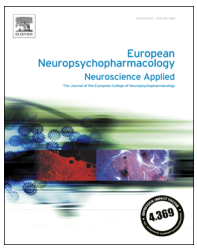

Leukocyte telomere length positively correlates with duration of lithium treatment in bipolar disorder patients

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Abstract
Bipolar disorder (BD) has been suggested to be associated with accelerated aging and premature cell senescence. While findings on shorter telomeres in BD are controversial, a recent study showed that long-term lithium treatment correlates with longer telomeres in BD. In our study, we sought to investigate the correlation between leukocyte telomere length (LTL) and long-term lithium treatment in a sample of 200 BD patients characterized for lithium response. We also compared data from two different methods commonly used to measure telomere length, quantitative PCR (qPCR) and quantitative fluorescence in situ hybridization (Q-FISH). We also measured, for the first time, the effect of lithium in vitro on the expression of the telomerase gene in human-derived neural progenitor cells (NPCs). Our findings showed that LTL correlated negatively with age ($p=0.0002$) and was independent of sex, diagnosis, age at onset, suicidal behavior, number of mood episodes, response to lithium and use of other psychotropic medications. After correcting for age, LTL was positively correlated with lithium treatment duration in patients treated for more than two years ($n=150$, $R=0.17$, $p=0.037$). There was a significant correlation between data measured with qPCR and Q-FISH ($p=0.012$,

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R=0.826). Lithium treatment increased telomerase expression in NPCs, though this effect was not statistically significant.
Our data support previous findings showing that long-term lithium treatment associates with longer telomeres in BD, though this effect appeared to be independent from clinical response to the treatment. Moreover, we suggested for the first time that lithium increases the expression of telomerase gene in human neural progenitor cells.

1. Introduction

Bipolar disorder (BD) is a disabling psychiatric illness characterized by alternating manic and depressive episodes, with a prevalence of 0.8–1.25 in the general population (Merikangas et al., 2007). Lithium is one of the most effective long-term treatments for BD. However, only 30% of patients treated with lithium present an excellent response with complete remission (Rybakowski, 2011). Despite many years of intensive research, the specific mechanisms by which lithium exerts its effects are not well understood. A large body of evidence has shown that lithium has neuroprotective effects through regulation of multiple signaling pathways (Rowe and Chuang, 2004). In particular, lithium has been reported to inhibit glycogen synthesis kinase-3β (GSK-3β) (Zhang et al., 2003), which, among others, is involved in apoptotic cell death (Beurel and Jope, 2006), and to up-regulate anti-apoptotic factors such as B-cell lymphoma protein-2 (Bcl-2), as well as brain-derived neurotrophic factor (BDNF) and β-catenin (Angelucci et al., 2000; Chen et al., 1999).

A recent study showed that long-term lithium treatment is associated with longer leukocyte telomere length (LTL) in BD (Martinsson et al., 2013). Even though several studies reported shorter telomeres in BD compared to healthy controls (Simon et al., 2006; Elvsåshagen et al., 2011; Rizzo et al., 2013; Lima et al., 2014), this finding has been put into question by a recent meta-analysis (Colpo et al., 2015). Nevertheless, BD seems to be associated with accelerated aging and cell senescence (Rizzo et al., 2014), a marker of which is telomere shortening. Interestingly, a recent study showed that lithium increases the expression of the gene codifying for the catalytic subunit of telomerase (*hTERT*) as well as its enzymatic activity in the hippocampus of a rat model of depression (Wei et al., 2015), further supporting the involvement of telomeres in mood disorders.

Telomeres consist of protective DNA-protein complexes containing TTAGGG tandem repeats located at the ends of chromosomes (Blackburn et al., 2006). This specialized end is crucial for integrity of the genome, preventing chromosome fusion and genomic instability (O'Sullivan and Karlseder, 2010). In most human cells, telomere repeats progressively shorten after each round of DNA replication and when they become critically short cells stop dividing or die for apoptosis (Calado, 2009). Longer telomere length (TL) has been associated to better survival (Bakaysa et al., 2007), family history of longevity (Atzmon et al., 2010) and healthy ageing (Njajou et al., 2009). In contrast, shorter TL has been reported in psychological stress and in age-related disorders, such as cardiovascular diseases and diabetes (Salpea et al., 2010; Saliques et al., 2010). In proliferative tissues, such as in germ cells, activated lymphocytes, developing neurons and in adult progenitor cells in general, where TL is essential for prolonged persistence and genetic stability (Flores et al., 2006, 2008), telomere shortening is counteracted by telomerase, a specialized reverse transcriptase (Osterhage and Friedman, 2009). In the human central nervous system, telomerase is abundant in neural progenitor cells in the developing (Klapper et al., 2001; Cai et al., 2002) and adult brain (Caporaso et al., 2003). However, in the other human tissues, telomerase is normally not expressed and telomeres shorten with age.

The majority of studies investigating TL in psychiatric disorders have focused on leukocyte telomere length (LTL), as this is recognized as a valuable and easily accessible marker of cellular aging, and is correlated with TL in other somatic cells (Bodelon et al., 2014).

Considering the paucity of findings on the effect of lithium on telomere dynamics in BD, we carried out a study aimed to investigate (a) the effect of long-term lithium treatment on LTL in BD, (b) the potential difference in LTL between patients responding and not responding to lithium as well as the impact of other clinical modifiers on LTL, and, for the first time, (c) the effect of lithium treatment on the expression levels of the catalytic subunit of human telomerase (hTERT) in human neuronal progenitor cells (NPCs).

2. Experimental procedures

2.1. Sample

The sample comprised 200 patients with BD (Table 1) of Sardinian ancestry for at least four generations, recruited at the Lithium Clinic of the Clinical Psychopharmacology Centre of the University Hospital of Cagliari, Italy from 1993 to 2012. Diagnosis was carried out by trained clinical psychopharmacologists according to DSM-IV criteria and the Schedule for Affective Disorder and Schizophrenia-LifetimeVersion (SADS-L) (Endicott and Spitzer, 1978). Age at onset, number of depressive, manic and hypomanic episodes, duration of illness, duration of lithium treatment and suicidal behavior were assessed. Use of other mood stabilizers, antidepressants, antipsychotics and benzodiazepines was also evaluated. Patients were characterized for lithium response using the “Retrospective Criteria of Long-Term Treatment Response in Research Subjects with Bipolar Disorder” as described previously (Grof et al., 2002; Manchia et al., 2013). This scale quantifies the degree of improvement in the course of treatment with a score from 0 to 10 (total score, TS). Patients with a TS equal to 7 or higher are considered lithium responders (LiRs). Our sample included 59 LiRs and 141 partial or non-responders (non-LiRs). We also measured TL using the quantitative fluorescence in situ hybridization (Q-FISH)